User Manual of SimBoolNet

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1. Introduction

SimBoolNet is an open source Cytoscape plugin for simulating the dynamics of signaling transduction using Boolean networks. Cytoscape is an open source software framework for visualization and analysis of molecular interaction networks (Shannon et al. 2003), and can be downloaded for free at http://www.cytoscape.org. In a Boolean network, every node is assigned a state of ON/OFF, and the state of a node is determined by the states of its up-stream neighboring nodes (i.e. nodes that are connected by an incoming edge). Given user-specified levels of stimulation to a signaling network, SimBoolNet simulates the responses of the downstream molecules. The process can be visualized with an animation and the results recorded for further analysis. SimBoolNet is a useful tool for hypothesizing causal relation and crosstalk among signaling molecules. A description of the algorithm behind SimBoolNet is given in (Zheng et al. 2009) and an experimental study using SimBoolNet has been reported in (Zielinski et al. 2009).

2. Installation

First, we assume that the user has installed Cytoscape on his or her computer and have an elementary level of experience using Cytoscape (instructions on how to install and use Cytoscape can be found at http://www.cytoscape.org). The SimBoolNet plugin can be downloaded for free at http://www.ncbi.nlm.nih.gov/CBBresearch/Przytycka/SimBoolNet. The current SimBoolNet package consists of two jar files, namely "SimBoolNet.jar" and "SimBoolNet_lib.jar". The latter jar file contains libraries for plotting simulation results.

To install SimBoolNet in Cytoscape platform as a plugin, please copy *both* "SimBoolNet.jar" and "SimBoolNet_lib.jar" files into the Cytoscape plugins folder (by default on Windows it is "C:\Program Files\Cytoscape_v2.6.3\plugins\" for version 2.6.3). Note that if the "SimBoolNet_lib.jar" file is not copied to the Cytoscape plugins folder, the plotting functionalities would not be available. The plugin will be automatically loaded when Cytoscape is launched. To uninstall the plugin, just delete the "SimBoolNet.jar" file from the plugin folder.

While, in this manual we describe the usage mainly under Windows environment, Cytoscape and the SimBoolNet plugin can be used under other operating systems (Linux, Mac OS X, etc.), since they were developed with the portal language of Java.

3. Preparing a network

Here we describe three different ways for preparing a network to be used with the SimBoolNet plugin. In particular, the users will be asked to specify the interaction types on the edges when setting up their networks. Please make sure that all edge interaction types are either "+" (activation) or "−" (blockage), because the plugin will ignore other symbols for the interaction types on edges.
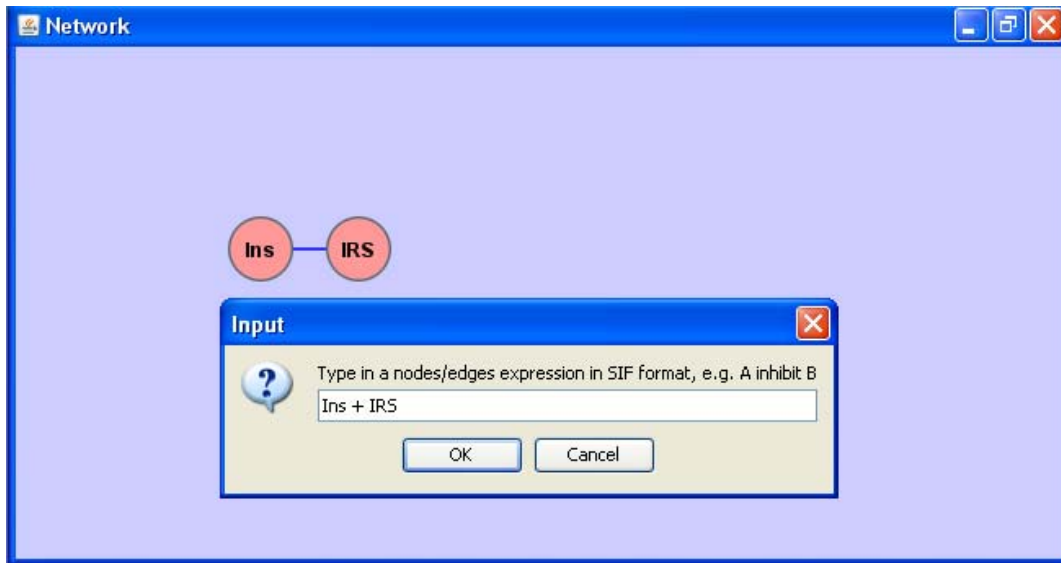
3.1. Creating a network

First, you can use the built-in Network Editor in Cytoscape to create a network from scratch. To do so, select "File->New->Network->Empty Network" to create an empty network. The Control Panel will automatically switch to the Editor tab (Fig. 3.1.1). From this tab, the user can drag nodes (pink circle) and edges (bold line) onto the network view.
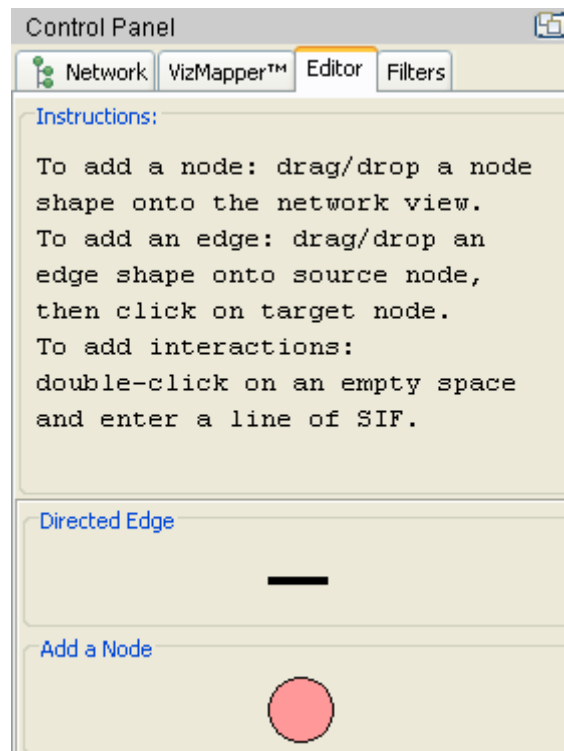
Figure 3.1.1. The Control Panel

Another way to add a node or an edge to a network is to double click on the network view to bring up an "Input" window (Fig. 3.1.2), where you can type in a node-node interaction in the "[source node] [edge interaction] [target node]" form.

Figure 3.1.2. Adding nodes and edges to a network

3.2. Importing a network from file

Alternatively, Cytoscape allows users to import networks from files from hard disk in various formats. In particular, we focus here on the delimited text and Microsoft Excel formats. The use of SimBoolNet is not affected by the specific formats of original files.

To import from a text or Microsoft Excel file, select "File->Import->Network from Table (Text/MS Excel)". In the window that appears (Fig. 3.2.1), you can load a file and then define the columns that refer to the source nodes, interactions, and target nodes.
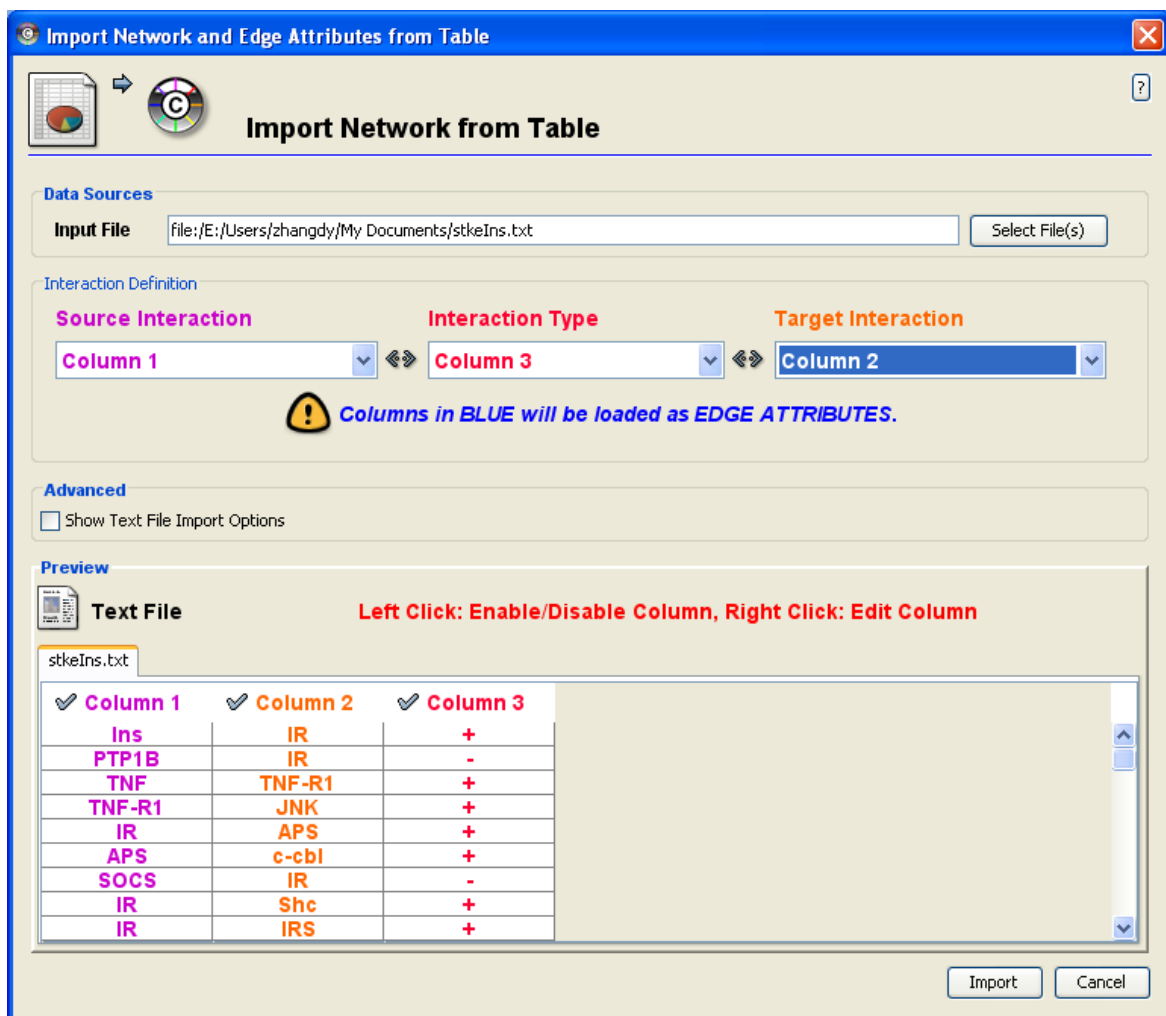
Figure 3.2.1. Importing a network from a text or Microsoft Excel file

3.2.1. Advanced Import Options (Delimiters)

When loading from a text file, the columns must be delimited by some character. By default, the import window uses tabs and spaces as delimiters, but your text file may use some other characters (e.g. comma or semicolon). To define your own delimiter(s), first check the "Show Text File Import Options" and then select the boxes for your particular delimiter(s) in the "Delimiter" area (Fig. 3.2.2).
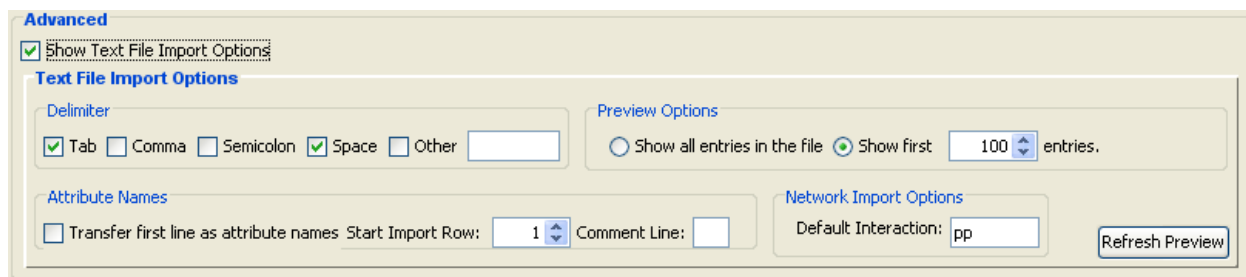
Figure 3.2.2. Advanced Import Options

Figure 3.2.3. A sample network shown in Notepad (.txt) and Microsoft Excel (.xls)

3.3. Saving and loading a session

If you have networks open in Cytoscape, then you can save your session to a .cys file by selecting "File->Save" from the menu. This session file contains the information from all networks that were open at the time the session was saved. To load a saved session, just choose "File -> Open" from the menu and locate the .cys file or simply double click the .cys file.

4. Using the Plugin

After installing the SimBoolNet plugin and preparing a network, you are now ready to use the plugin. At the bottom of the "Data Panel", you will find a tab labeled "SimBoolNet". Click the "SimBoolNet" tab and you can access the control buttons of the plugin (Fig. 4.0.1).
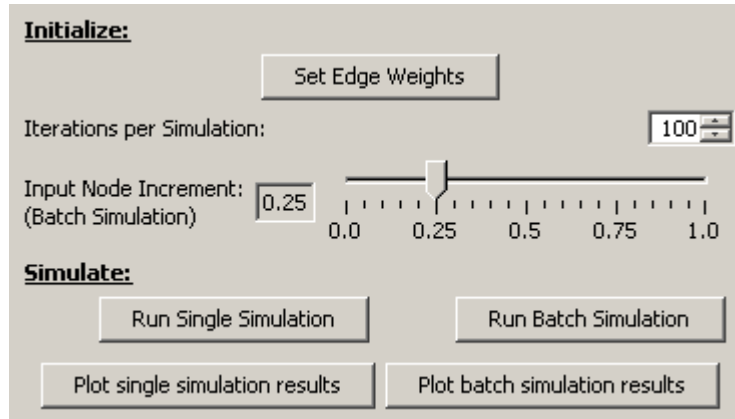
Figure 4.0.1. The control panel of SimBoolNet plugin

4.1. Initialization

4.1.1. Set Edge Weights

You can set edge weights for individual edges by clicking on the "Set Edge Weights" button in the SimBoolNet panel. The default edge weight is 0.8.

The Set Edge Weight window (Fig. 4.1.1) shows a list of all edges in the network, along with sliders in order to set individual edge weights. You can also click on the check boxes next to each edge name in order to modify multiple edge weights simultaneously. The "Select all" and "Deselect all" buttons make selecting or deselecting multiple edges easier, by selecting or deselecting every edge in the list. The two sliders on the bottom of the window will edit all selected activation or blockage edge weights, respectively.
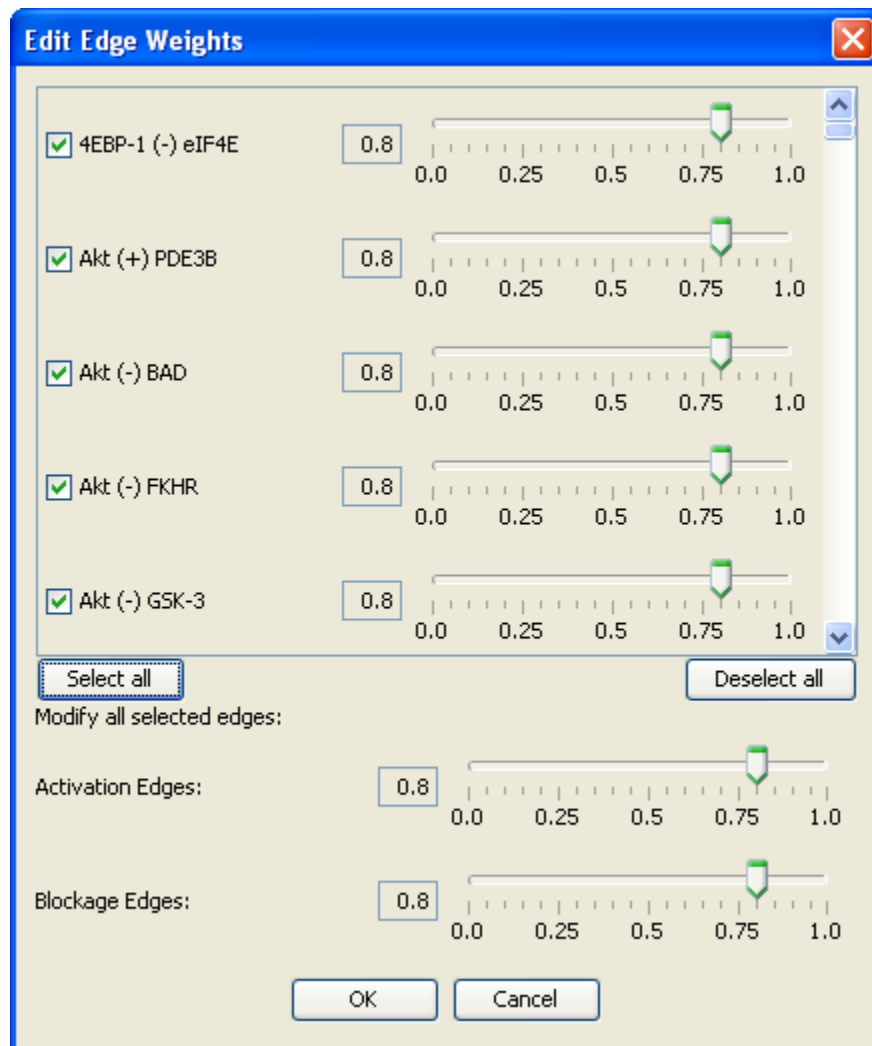
Figure 4.1.1. The Edit Edge Weights window

4.1.2. Iterations per Simulation

This option defines how many iterations a simulation will run before outputting results. More iterations will take longer to run but can provide more accurate results. By default, this is set to 100, but the user can specify any positive integer.

4.1.3. Input Node Increment (Batch Simulation)

This option defines how much the input level of input nodes will increase per simulation when running a batch-mode simulation, the meaning of which will be explained in Section 4.2.2. The default value is 0.25, but it can be set to any number between 0 and 1.
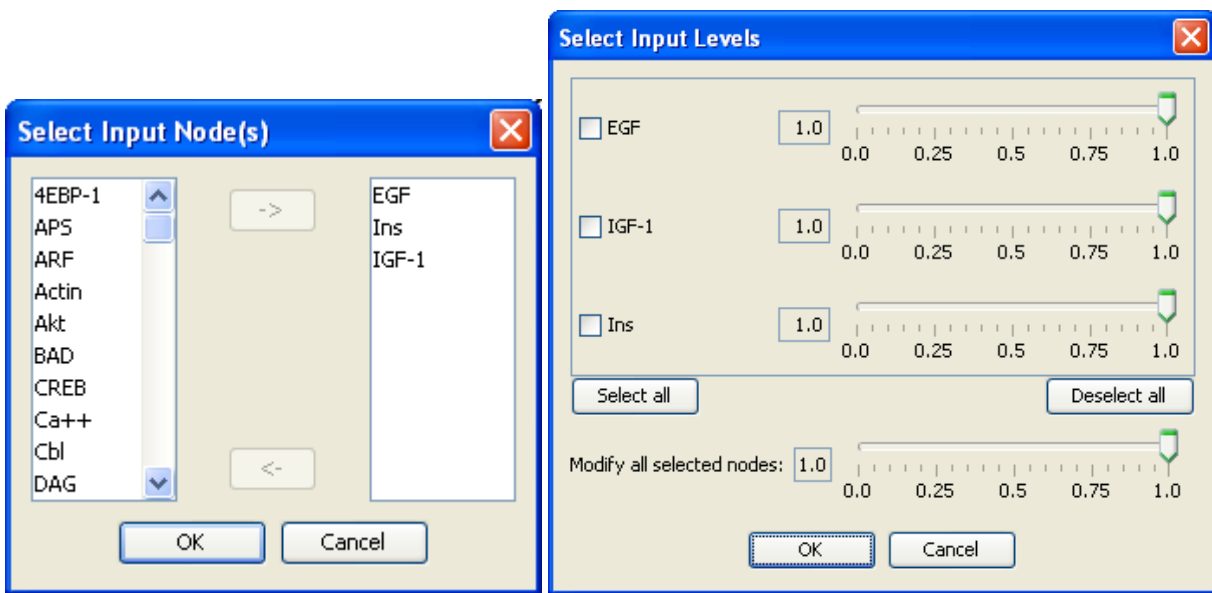
4.2. Running Simulations

4.2.1. Single Simulation

To run a single simulation, click on the button labeled "Run Single Simulation" in the SimBoolNet panel.

A window will appear for you to select the input nodes from a list of all nodes in the network (Fig. 4.2.1 (a)). To add a node to the input list (right side), you can click on a node from the available list (left side), and then click the "->"button, and the chosen node will appear on the right. . To unselect a node from the input list, use the "<-" button. You can also select multiple nodes (by holding Ctrl or Shift) and the click on the respective arrow buttons to move them between lists. The plugin will remember nodes you have previously set as input nodes. Note that both lists will initially be sorted alphabetically, but nodes will be added to the bottom when moving between lists. In the end, click the "OK" button to finish the selection of input nodes.

Next, a second window will appear for you to set the input levels for the selected input nodes (Fig. 4.2.1 (b)). The sliders function similarly to the sliders in the "Set Edge Weights" window (see Section 4.1.1).


Figure 4.2.1. The windows for (a) selecting input nodes and (b) setting input levels.

The plugin then starts the simulation with the input network and parameter values. As the simulation is running, the plugin provides a simple animation to visualize node activities (Fig. 4.2.2). This is shown as node color changing: grey represents low activity, while red represents high activity. Also, the visual style will be altered during the animation; edge widths will reflect weight and edge heads will reflect the interaction types (arrows for "+" and "T" shape lines for "-").
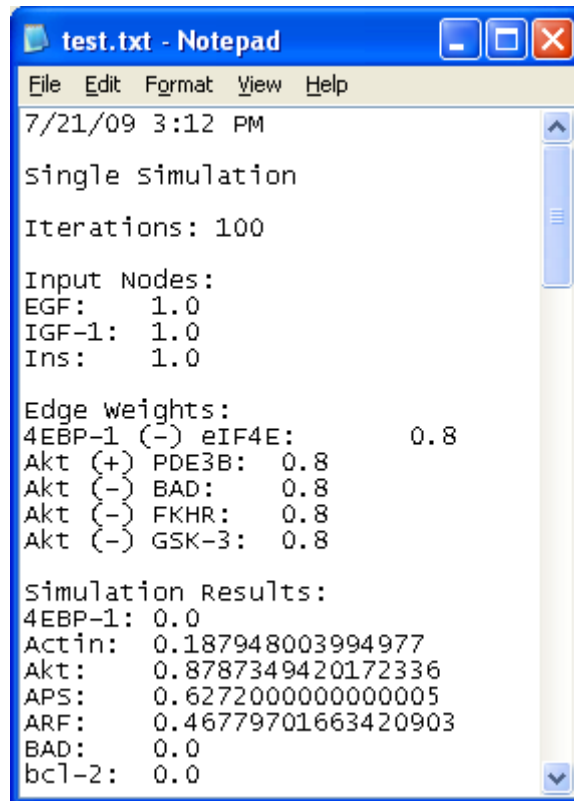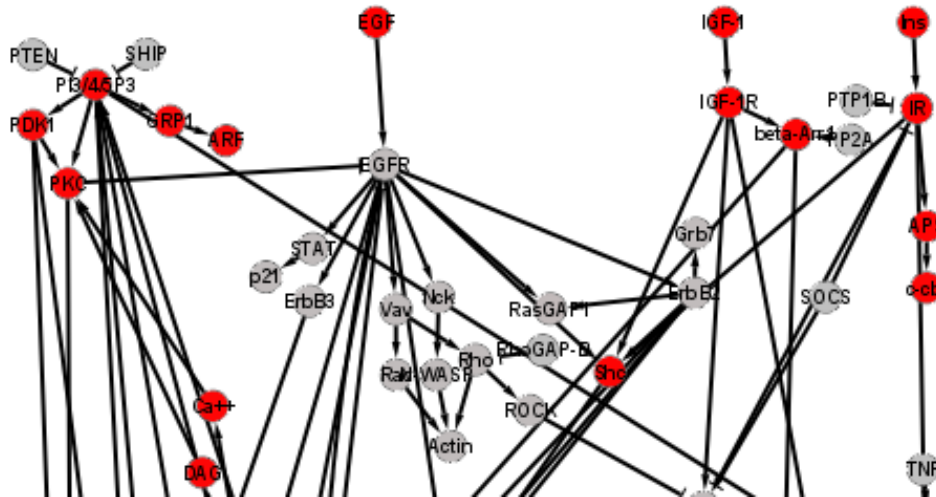
Figure 4.2.2. A portion of a sample network after running a simulation

After the simulation is completed, a window will appear asking if you want to view the results of the simulation. If you choose "Yes", you will be provided with three sets of information: (1) Input Nodes and their input levels (Fig. 4.2.3 (a)), (2) Edge Weights (Fig. 4.2.3 (b)), and (3) Simulation Results (Fig. 4.2.3 (c)).
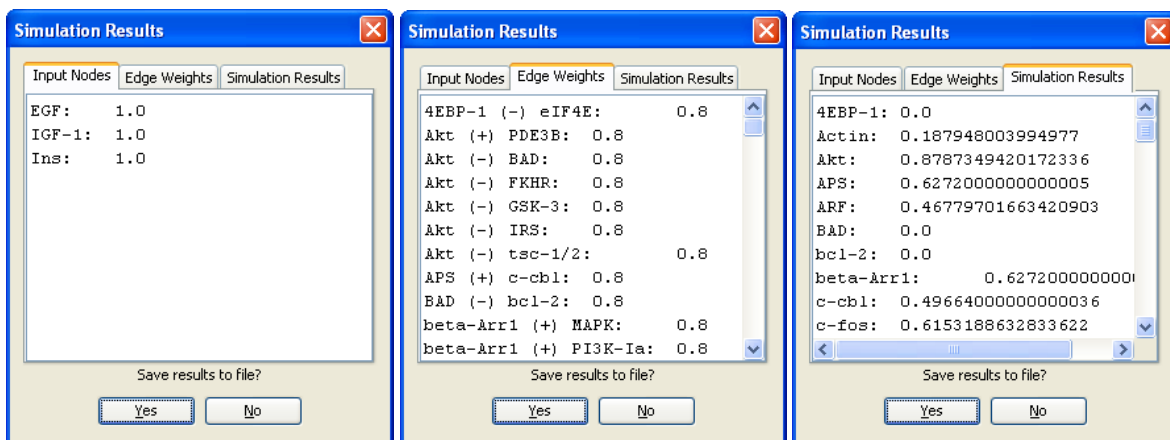
Figure 4.2.3. The Simulation Results window, showing (a) Input Nodes tab, (b) Edge Weights tab, and (c) Simulation Results tab.

You can then save the results to a file. If you select a file that already exists, you are given the option to overwrite or append to the end. The plugin will record the following information in order: date and time when the simulation finishes, the simulation mode (Single or Batch), the number of iterations, input nodes and stimulation levels, edge weights, and simulation results (Fig. 4.2.4).

Figure 4.2.4. Sample output file of a Single Simulation (omitting many weights and results).
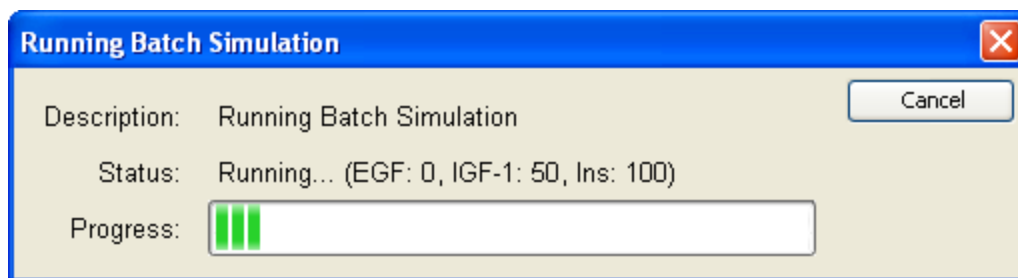
4.2.2. Batch Simulation

A batch simulation is a series of single simulations in which the plugin will automatically adjust the input levels for the input nodes by a user-specified amount (as defined by "Input Node Increment" in Section 4.1.3). The plugin will keep track of each simulation's results for a selection of output nodes and report the outputs after the batch has been completed. To run a batch simulation, click on the "Run Batch Simulation" button in the SimBoolNet panel.

First, a window will appear for selecting input nodes (Fig. 4.2.5 (a)), followed by a second window to select output nodes (Fig. 4.2.5 (b)). These windows are similar to the "Select Input Node(s)" window for the Single Simulation (see Section 4.2.1) and the lists can be manipulated in the same way.
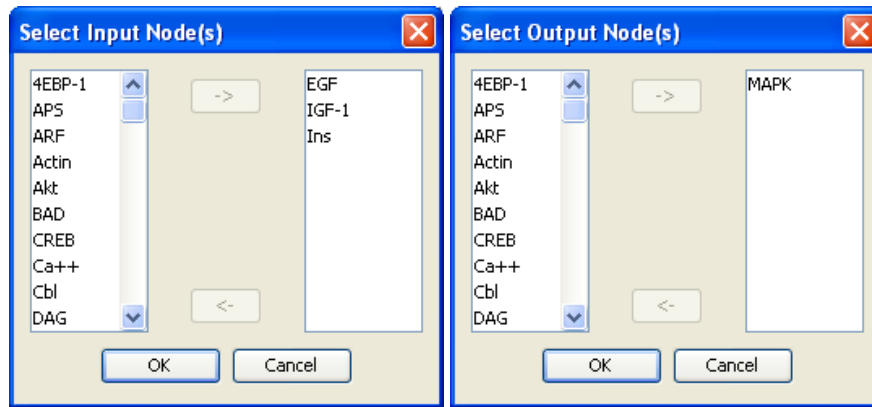
Figure 4.2.5. (a) Select Input Node(s) and (b) Select Output Node(s) windows.

After selecting input and output nodes, the plugin will run a series of simulations. There will not be an animation, but a progress bar will tell you the status of the simulations (Fig. 4.2.6), as well as allow you to cancel it.


Figure 4.2.6. The Batch Simulation progress bar.

Once completed, the plugin will ask if you want to view results. If you select "Yes", it will show a table in which each row contains the activity levels of input and output nodes for a single simulation (Fig. 4.2.7).
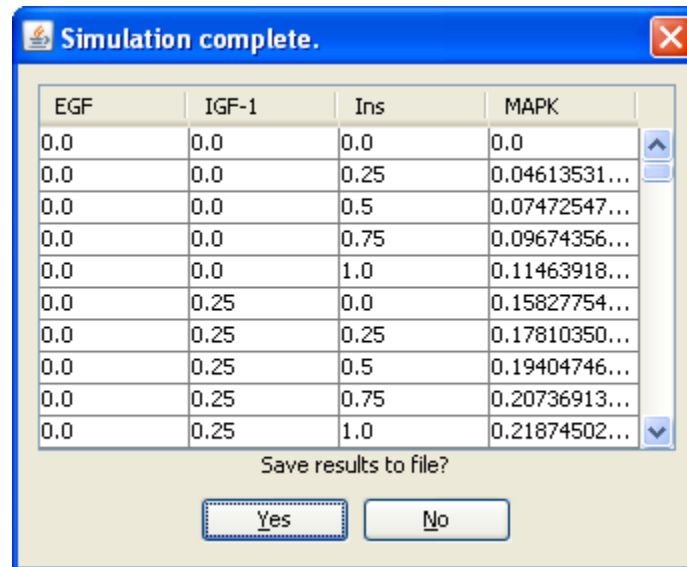
Figure 4.2.7. Results of a Batch Simulation

As with Single Simulation, you can save these results to file. The plugin will record the following information in order: date and time of the simulation, type of simulation (Single or Batch), amount of iterations per simulation, edge weights, and simulation results (Fig. 4.2.8).
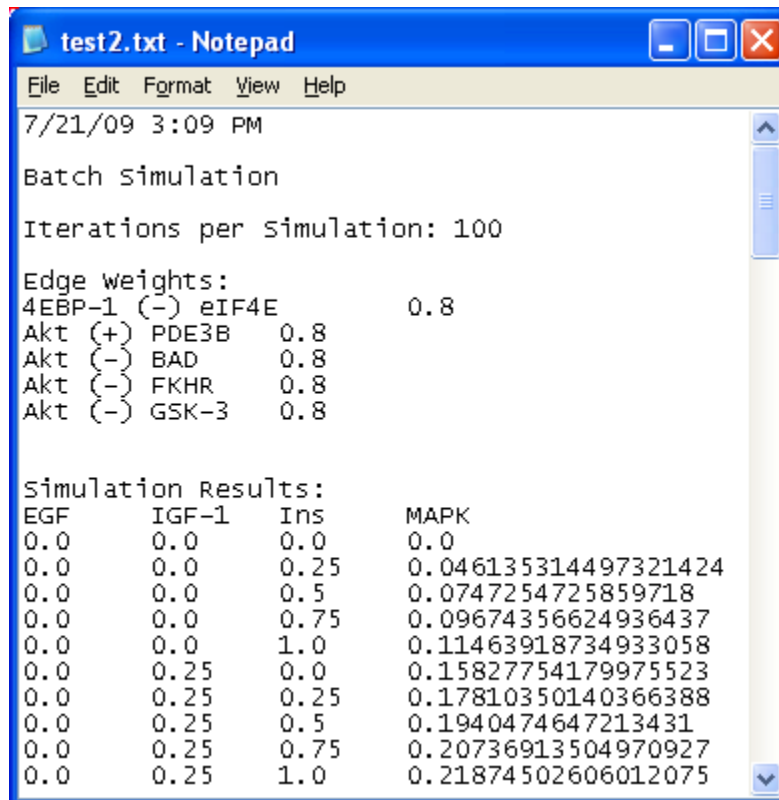

Figure 4.2.8. Sample output file of a Batch Simulation (including edge weights and results)

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4.3. Visualizing Simulation Results

To facilitate the analysis of simulation results, SimBoolNet generates charts for both single and batch simulations, using the Java library of JFreeChart (Gilbert 2009). At the bottom of SimBoolNet control panel (Figure 4.0.1), there are two buttons with labels "Plot single simulation results" and "Plot batch simulation results". After finishing a single or batch simulation, you can launch a dialog box with a canvas for plotting simulation results by clicking one of the buttons.

4.3.1. Time Series from Single Simulation

After clicking the "Plot single simulation results" button, you will see a panel similar as Figure 4.3.1 (although the chart is still empty). This is to plot time series recording the dynamic changes of node activities during the single simulation.
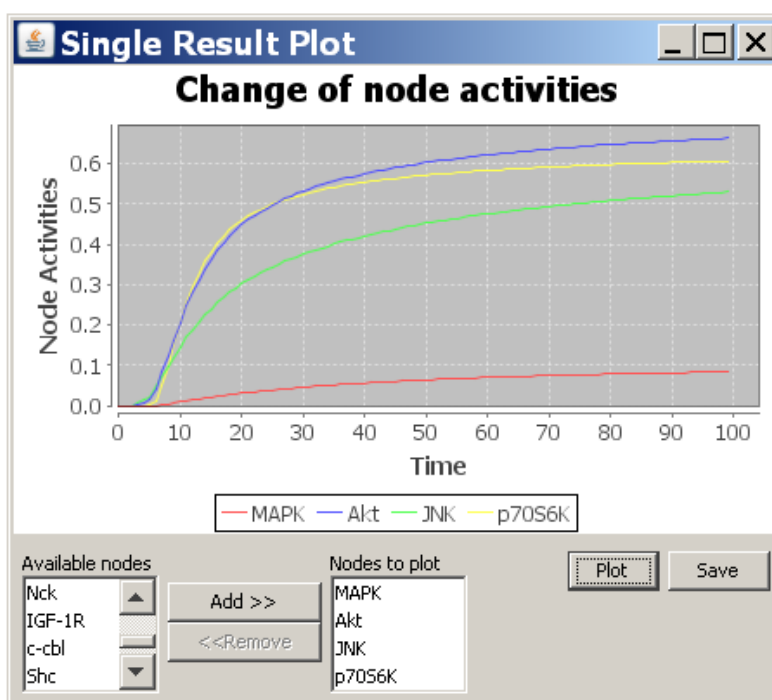
Figure 4.3.1. Time series of changes of node activities from a single simulation

First, let us select a list of nodes to be plotted. At the bottom left corner of the panel, we can select one or multiple nodes from the list of "Available nodes" by mouse clicking. By clicking "Add>>" button, the selected nodes will appear on the right list under "Nodes to plot". You can remove nodes to plot by selecting the latter list and clicking "<<Remove" button. This is similar to how we select input nodes in single or batch simulation (Figure 4.2.1 and Figure 4.2.5).

Then, we simply click the "Plot" button and the time series will be displayed on the chart at the top. Each node will be drawn with a distinct color as denoted at the bottom of the chart. You can change the

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list of "Nodes to plot" using "Add>>" and "<<Remove" buttons, and then click the "Plot" button to get a new chart.

For more detailed analysis, you can click the "Save" button to export the time series to a text file. Each row of the file is a time series for a node, formatted as the name of the node followed by a list of numbers (representing node activities) delimited by commas.

4.3.2. Scatter Plot and Regression Lines from Batch Simulation

Click the "Plot batch simulation results" button on SimBoolNet's control panel, and you will see a panel as in Figure 4.3.2. This panel is used to plot the scatter plots and regression lines between the activities of input and output nodes. The data are from a batch simulation as shown in the table of Figure 4.2.7. You are advised to save the data to a text file once the batch simulation is finished.

A batch simulation consists of a list of single simulations for different combinations of activity levels of multiple input nodes. However, the 2-dimensional chart in this panel can have only one predictor variable at the horizontal axis. Thus we first need to fix the input levels of input nodes other than the predictor node, which is called "marginal control". To do that, you can select a level for each node in the list of combo boxes under "Marginal control" label. The levels are specified in the batch simulation by the "Input Node Increment" (see Section 4.1.3).
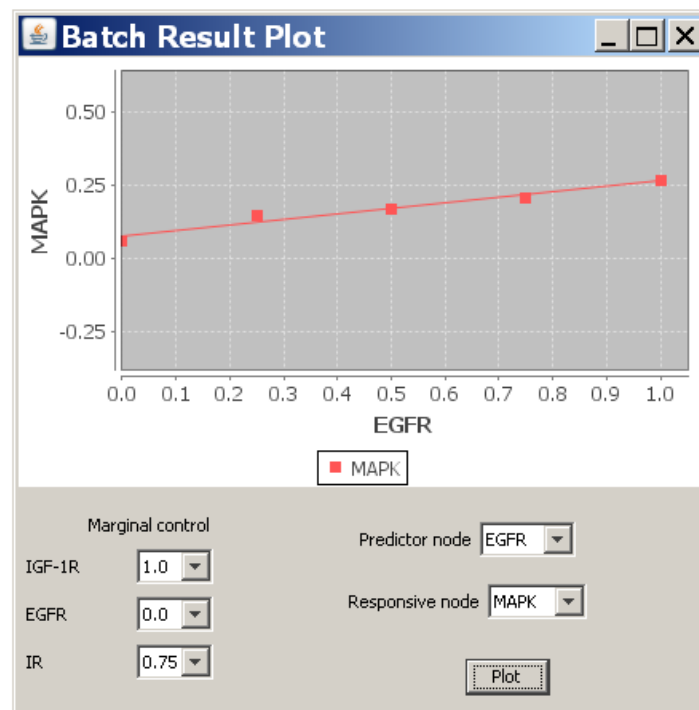
Figure 4.3.2. A scatter and line plot of causal relation between two nodes from a batch simulation

The second step is to select the predictor and responsive nodes from input and output nodes of batch simulation, which will be the x-axis and y-axis of the scatter plot. Note that, if an input node is selected as the predictor, the marginal control for that node will be automatically disabled.

Once you have selected the predictor and responsive nodes, you can click the "Plot" button to see the scatter plot of the two nodes. A linear regression line is also generated to fit the scatter points. You can change the marginal control levels to see how the trend will change. This is useful to study say the crosstalk between cellular signaling pathways. For more detailed analysis, you can use the text file containing batch simulation results (Figure 4.2.8).

References

Gilbert D (2009) The JFreeChart Class Library. Available: http://www.jfree.org/jfreechart/.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11): 2498-2504.

Zheng J, Zhang D, Przytycki PF, Zielinski R, Capala J et al. (2009) SimBoolNet – A Cytoscape Plugin for Dynamic Simulation of Sig-naling Networks. Submitted.

Zielinski R, Przytycki PF, Zheng J, Zhang D, Przytycka TM et al. (2009) The crosstalk between EGF, IGF, and Insulin cell signaling pathways - computational and experimental analysis. BMC Systems Biology 3(88).